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A SIMPLE METHOD FOR ISOLATION OF INFLUENZA BACILLUS

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The great difficulty in the isolation of the influenza bacillus from the respiratory tract is due to the fact that the accompanying bacteria usually overgrow and obscure the organism. This statement is borne out by the first studies that we made of cultures taken from the tonsils and the nasopharynx of 12 patients suffering from influenza or influenza accompanied with pneumonia. The material obtained was streaked on human blood-agar plates, and though colonies of the influenza bacillus developed in all but one, the subsequent isolation in pure culture was extremely difficult, none being pure on the first subculture.

We also examined the sputums from 59 cases of pneumonia following influenza, and though we were able to demonstrate the presence of the influenza bacillus in mixture with other organisms by direct smears in 15 instances, but 10 showed these organisms in the primary culture and only one was obtained pure in the first subculture.

These discouraging results led us to seek for a method which would favor the growth of the influenza bacillus in the presence of accompanying bacteria.

In 1912 Churchman demonstrated the selective action of gentian-violet on bacteria. He showed that agars containing this dye in strengths of 1: 100,000 to 1: 1,000 were either inhibitive or destructive to gram-positive organisms, while gram-negative bacteria were not affected. With this in mind we developed a method for obtaining the influenza bacillus in pure culture from mixtures containing it.

The usual accompanying organisms in the naso-pharynx, trachea or lungs are the pneumococcus, streptococcus and staphylococcus, all of which are gram-positive and therefore inhibited by gentian-violet.

After many experiments with blood-agar containing varying amounts of the dye, we found that a concentration of 1: 5,000 gave the best results. Agar was prepared with a H ion of 7.1 and a gentian-violet content of 1: 5,000 — taking a concentrated alcoholic solution of the dye as unity. At first we prepared our medium by adding to the melted and cooled gentian-violet-agar 25% of sterile ascitic fluid and 5% of sterile defibrinated human blood. We soon found that the serum interfered with the inhibiting action of the gentian-violet for the gram-positive organisms. It was therefore eliminated.

TABLE 1

Showing the Advantage of Using Gentian-Violet-Blood-Agar in Isolating the Influenza Bacillus

No.	Gram Stain	Blood-Agar		Gentian-Violet Blood-Agar	
		Primary Culture	Subculture	Primary Culture	Subculture
1	Influenza bacillus; G.+ chains	Hemolytic streptococcus; influenza bacillus	Influenza bacillus, pure	Influenza bacillus; streptococcus	Influenza bacillus, pure
2	Influenza bacillus; G.+ diplococci and chains	Pneumococcus; streptococcus	Influenza bacilli not present	Influenza bacillus; streptococcus; pneumococcus	Influenza bacillus, pure
3	G.+ diplococci and chains; influenza bacillus	Hemolytic streptococcus; pneucoccus; influenza bacillus (few)	Influenza bacillus, not pure	Influenza bacillus; streptococcus; pneumococcus	Influenza bacillus, pure
4	Influenza bacillus; G.— large bacillus; G.+ diplococcus	Pneumococcus Type IV; bacillus lactis aerogenes; influenza bacillus	Influenza bacillus, not pure	Bacillus lactis aerogenes; influenza bacillus; pneumococcus	Influenza bacillus, pure
5	G.+ diplococci and clusters influenza bacillus	Staphylococcus albus; pneumococcus mucosus Type III; influenza bacillus	Influenza bacillus, not pure	Influenza bacillus; pneumococcus; staphylococcus	Influenza bacillus, pure
6	G.+ diplococcus; influenza bacillus	Pneumococcus; influenza bacillus	Influenza bacillus, not pure	Influenza bacillus; pneumococcus	Influenza bacillus, pure
7	G.+ diplococci; chains and clusters; influenza bacillus	Pneumococcus Type IV; staphylococcus albus; influenza bacillus	Influenza bacillus, not pure	Influenza bacillus; pneumococcus; staphylococcus	Influenza bacillus, pure
8	G.+ diplococci and chains; influenza bacillus	Pneumococcus; hemolytic streptococcus; staphylococcus albus	Influenza bacilli not present	Pneumococcus; staphylococcus; influenza bacillus; streptococcus	Influenza bacillus, pure
9	G.+ diplococci and chains; influenza bacillus	Hemolytic streptococcus; influenza bacillus; pneumococcus	Influenza bacillus, not pure	Influenza bacillus; streptococcus; pneumococcus	Influenza bacillus, pure

The gentian-violet blood-agar was poured into sterile plates and stored for use in the refrigerator. Surface streaks were made from washed sputum or from the naso-pharynx by means of tubes.

While the pneumococcus, streptococcus and staphylococcus were not entirely eliminated from the primary plate, they were as a rule so much inhibited in their growth that influenza bacillus colonies were easily seen and recognized. Control plates on plain blood agar were often so overgrown by the accompanying bacteria as to make the isolation of the influenza bacillus impossible.

Subcultures on gentian-violet blood-agar usually completely inhibited associated gram-positive organisms. If still present, a second subculture on the same medium produced a pure culture of influenza bacillus. As shown by Table 1, we cultured 9 sputums in duplicate — on plain blood-agar and on gentian-violet blood-agar. In one case the influenza bacillus was isolated by both methods in the same generation and in the other 8 cases it was obtained pure in the second or third generation only on gentian violet plates, and in only a few instances in later subcultures on plain blood plates.

SUMMARY

By the employment of gentian violet blood agar the isolation of the influenza bacillus is comparatively easy. The use of this medium gives a much higher percentage of positive results than plain blood agar.